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**Term:** 6194548.pn.

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| L4 | L3 and E222G | 0 | L4 |
| L3 | 6194548.pn. | 1 | L3 |
| L2 | l1 and E222G | 1 | L2 |
| L1 | 6124128.pn. | 1 | L1 |

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L2: Entry 1 of 1 File: USPT Sep 26, 2000

DOCUMENT-IDENTIFIER: US 6124128 A
TITLE: Long wavelength engineered fluorescent proteins

US Patent No. (1):
6124128

Detailed Description Text (5):
In another aspect, this invention provides a nucleic acid molecule comprising a nucleotide sequence encoding a functional engineered fluorescent protein whose amino acid sequence is substantially identical to the amino acid sequence of Aequorea green fluorescent protein (SEQ ID NO:2) and which differs from SEQ ID NO:2 by at least an amino acid substitution at L42, V61, T62, V68, Q69, Q94, N121, Y145, H148, V150, F165, I167, Q183, N185, L220, E222 (not E222G), or V224, said functional engineered fluorescent protein having a different fluorescent property than Aequorea green fluorescent protein. In one embodiment, amino acid substitution is:

Detailed Description Text (122):
The results of previous random mutagenesis have implicated several amino acid side chains to have substantial effects on the spectra and the atomic model confirms that these residues are close to the chromophore. The mutations T203I and E222G have profound but opposite consequences on the absorption spectrum (T. Ehrig et al. FEBS Letters 367:163-166 (1995)). T203I (with wild-type Ser.sup.65) lacks the 475 nm absorbance peak usually attributed to the anionic chromophore and shows only the 395 nm peak thought to reflect the neutral chromophore (R. Heim et al. Proc. Natl. Acad. Sci. USA 91:12501-12504 (1994); T. Ehrig et al. FEBS Letters 367:163-166 (1995)). Indeed, Thr.sup.203 is hydrogen-bonded to the phenolic oxygen of the chromophore, so replacement by Ile should hinder ionization of the phenolic oxygen. Mutation of Glu.sup.222 to Gly (T. Ehrig et al. FEBS Letters 367:163-166 (1995)) has much the same spectroscopic effect as replacing Ser.sup.65 by Gly, Ala, Cys, Val, or Thr, namely to suppress the 395 nm peak in favor of a peak at 470-490 nm (R. Heim et al. Nature 373:664-665 (1995); S. Delagrave et al. Bio/Technology 13:151-154 (1995)). Indeed Glu.sup.222 and the remnant of Thr.sup.65 are hydrogen-bonded to each other in the present structure, probably with the uncharged carboxyl of Glu.sup.222 acting as donor to the side chain oxygen of Thr.sup.65. Mutations E222G, S65G, S65A, and S65V would all suppress such H-bonding. To explain why only wild-type protein has both excitation peaks, Ser.sup.65, unlike Thr.sup.65, may adopt a conformation in which its hydroxyl donates a hydrogen bond to and stabilizes Glu.sup.222 as an anion, whose charge then inhibits ionization of the chromophore. The structure also explains why some mutations seem neutral. For example, Gln.sup.80 is a surface residue far removed from the chromophore, which explains why its accidental and ubiquitous mutation to Arg seems to have no obvious intramolecular spectroscopic effect (M. Chalfie et al. Science 263:802-805 (1994)).